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(54) Title: PEPTIDES COMPRISING A T-CELL EPITOPE SPECIFIC TO COLLAGEN II

(57) Abstract

The invention provides an isolated peptide comprising an amino acid sequence of formula (I): A_1-Xaa-Gly-A_4-A_5-Gly-A_7-Xaa-Gly, wherein A_1 represents an amino acid residue with an aromatic or aliphatic side chain, A_4 represents an asparagine or arginine residue or an amino acid residue with an aromatic or aliphatic side chain, A_5 represents any naturally occurring amino acid, A_7 represents an amino acid with a negatively charged residue, Xaa represents any amino acid residue, and Gly represents a glycine residue; and the use of such peptides in medical therapy, particularly in the treatment of autoimmune conditions such as rheumotoid arthritis.

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PEPTIDES COMPRISING A T-CELL EPITOPE SPECIFIC TO COLLAGEN II

The present invention provides new peptides derived from collagen CII, methods for their preparation and their use in medical therapy, particularly in the treatment of rheumatoid arthritis and related autoimmune conditions

Collagen molecules are some of the main structural proteins of connective tissue. They consist of three polypeptide chains forming an extended triple helical structure with a unique X-Y-Gly repetitive amino acid sequence. The extracellular matrix of cartilage is unique in containing collagens mainly of type II, but also of types IX and XI. CII has recently been cloned from the mouse and its entire sequence of 1419 amino acids determined (Metsarantam et al., 1991, J Biol Chem, 266: 16862-9).

Type II collagen is thought to be "hidden " from cells of the immune system as it is only found in avascular tissues, such as the cartilage, and the vitreous body of the eye. The immune system is therefore not completely tolerant of its own type II collagen As a sequestered protein, CII has the ability, when injected in Freund's complete adjuvant, to induce tissue-specific autoimmune disease. The disease that it induces is termed collagen induced arthritis (CIA). CIA is normally induced by injection of foreign CII, which provokes the production of T cells and antibodies able to recognise the corresponding self-protein.

Susceptibility to CIA in mice is limited to MHC types I-A^q and I-A^r. The binding motifs of these molecules are similar to those of human DR4 and DR1. Therefore it is believed that CII is an autoantigen in rheumatoid arthritis(RA).

No causal therapy is currently available for RA. Existing treatment methods e.g. application of corticosteroids, are unspecific, as they suppress the immune response in

general. Current research suggest an important role for autoreactive T cells in the pathogenesis of RA which has lead to the concept that tolerization of these pathogenic T cells by nasal/oral administration of immunogenic peptides might be of therapeutic potential.

Miyahara, H. et al (Immunology 1995 86 110-115) describe the use of a fragment of type II collagen in the suppression of arthritis in mice. The fragment used (CII 607-621) does however not contain a binding motif to any of the HLA-DR molecules associated with RA in man and would therefore not be effective for use as a toleragen in human RA therapy.

WO 96/20950 purports to describe type II collagen peptides capable of binding to the human HLA DRB1 MHC protein which it is suggested would be of use in RA therapy. However these peptides which are present in the 273-404 region of collagen CII protein show only weak activity in relevant assays for putative toleragens for RA . The peptides of the present invention are of a significantly greater therapeutic efficacy.

The present invention provides peptides which have been found to be particularly effective in inducing immune tolerance to collagen CII derived T-cell epitopes. These peptides may be used in the treatment of autoimmune conditions such as rheumatoid arthritis.

There is therefore provided according to the present invention, an isolated peptide having, or comprising, an amino acid sequence of formula (I):

$$A_1\text{-Xaa-Gly-}A_4\text{-}A_5\text{-Gly-}A_7\text{-Xaa-Gly} \qquad (I)$$

wherein;

A_1 represents an amino acid residue with an aromatic or aliphatic side chain,

A_4 represents an asparagine or arginine residue or an amino acid residue with an aromatic or aliphatic side chain,

A_5 represents any naturally occurring amino acid,

A_7 represents an amino acid with a negatively charged residue,

Xaa represents any amino acid residue, and

Gly represents a glycine residue.

Peptide of the present invention are preferably 9, 10 , 11, 12, 13, 14 or 15 amino acid peptides; and are more preferably 9 amino acid peptides.

In peptides of the present invention the following independent preferences apply;

-A_1 is F, I, L, A or P and is most preferably F

-A_4 is F, I, L, A, R, N or P and is most preferably F

-A_5 is K or R

-A_7 is E , D, Q, P or N and is most preferably Q.

Preferred peptides according to the present invention include those comprising, or having, one of the following sequences:

ESG SPG ENG, PPG ADG QPG, ARG NDG QPG, QPG AKG DQG, APG AKG EAG, PTG VTG PKG, AQG SRG EPG, RVG PPG ANG, PAG ASG NPG, ANG NPG PAG, TDG IPG AKG, DPG LQG PAG, SAG APG IAG, APG EKG EPG, IAG APG FPG, PQG LAG QRG, FPG PRG PPG, PKG ANG DPG, APG ASG DRG, LPG ARG LTG, DAG PQG KVG, ALG APG APG, PAG ANG EKG, KQG DRG EAG, or ARG APG EPG (SEQ IDs Nos 1 to 25 respectively); and more preferably either RVG PPG ANG (SEQ ID No 8) or ANG NPG PAG (SEQ ID No 10).

Preferred examples of peptides according to the invention also include those comprising, or having, one of the following amino acid sequences:

VKG ESG SPG ENG SPG; FAG PPG ADG QPG AKG; AAG ARG NDG QPG PAG; ADG QPG AKG DQG EAG; APG AKG EAG PTG ARG; PQG PTG VTG PKG ARG; PEG AQG SRG EPG NPG; AAG RVG PPG ANG NPG; PAG ASG NPG TDG IPG; PPG

ANG NPG PAG PPG; NPG TDG IPG AKG SAG; RAG DPG LQG PAG APG; AKG SAG APG IAG APG; PAG APG EKG EPG DDG; APG IAG APG FPG PRG; PPG PQG LAG QRG IVG; APG FPG PRG PPG PQG; LAG PKG ANG DPG RPG; KQG APG ASG DRG PPG; EPG LPG ARG LTG RPG; RPG DAG PQG KVG PSG; ETG ALG APG APG PPG; PPG PAG ANG EKG EVG; PTG KQG DRG EAG AQG; or STG ARG APG EPG ETG (SEQ IDs Nos 26 to 52 respectively); more preferably AAG RVG PPG ANG NPG (SEQ ID No 33), and most preferably PPG ANG NPG PAG PPG (SEQ ID No 35).

Preferably the peptide according to the invention comprises a 9 to 15 amino acid sequence present uninterrupted in the 701-721 region of collagen II.

The invention also relates to a peptide comprising a T-cell epitope specific to collagen II which peptide comprises at least nine amino acids having the same sequence as and selected consecutively from the 110-239, 338-379 and 587-895 portions of the sequence of collagen II wherein each amino acid is optionally replaced by a functionally equivalent amino acid and wherein the peptide is of formula I as defined above.

According to the invention there is further provided a pharmaceutical composition comprising a peptide according to the invention in association with a pharmaceutically acceptable carrier or diluent. The compositions are preferably for use in providing tolerance against an autoimmune condition such as rheumatoid arthritis and relapsing polychondritis.

The invention further provides the use of a peptide according to the invention or of a composition according to the invention in the manufacture of a medicament for use in the treatment of an autoimmune condition.

According to the invention there is also provided a method of treating a human or animal suffering from an autoimmune condition such as rheumatoid arthritis and relapsing

polychondritis, which method comprises supplying the human or animal with a therapeutically effective amount of the peptide or of the composition.

The peptides according to the invention may be prepared using methods known to the skilled man. For example by using the standard solid phase sequential coupling technique utilising an automatic peptide synthesiser (see for example: Jones, J. The Chemical Synthesis of Peptides, pp 132-156, first edition, Oxford University Press, 1991 and R. Epton (ed) Innovation and Perspectives in Solid Phase Peptide Synthesis, SPCC (UK), Ltd, 1990). The preparation starts from the C-terminal amino acid which can be obtained grafted to a methylbenzhydrylamine, benzhydrylamine or chloromethylated resin or a suitable solid support. The other amino acids are grafted step by step, after having protected the side chains thereof. In this coupling method the alpha-amino groups of the amino acids are protected with F-moc or t-Boc methodology. Protective groups for the side chains of amino acids are well known in the art. The whole protected peptide is released from the chloromethylated resin by ammoniolysis to obtain the protected amide, or from the methylbenzhydrylamine or benzhydrylamine resins by acidolysis.

Peptides according to the invention may also be prepared using solution methods, by either stepwise or fragment condensations (see for example: Jones, J. The Chemical Synthesis of Peptides, pp 115-131, first edition, Oxford University Press, 1991). An appropriately alpha aminoprotected amino acid is coupled to an appropriately alpha carboxyl protected amino acid (such protection may not be required depending on the coupling method chosen) using diimides, symmetrical or unsymmetrical anhydrides, or other coupling reagents or techniques known to those skilled in the art. These techniques may be either chemical or enzymatic. The alpha amino acid an/or alpha carboxyl protecting groups are removed and the next suitably protected amino acid or block of amino acids are coupled to extend the growing peptide. Various combinations of protecting groups and of chemical and/or enzymatic techniques and assembly strategies can be used in each synthesis.

The peptides according to the invention are defined as comprising a T-cell epitope. In other words they are capable of activating T-cells. There are several known techniques for determining binding strength . Preferably a peptide according to invention can activate the T-cells with a binding strength of at least 2 in an IFN-γ release assay and/or with a stimulation index of at least 3. The IFN-γ release assay can be carried out using methods known in the art or by using the methodology described in the Examples herein. Similarly the stimulation index can be determined using known proliferation assays, for example those described in "Analysis of type II collagen reactive T cells in the mouse" Andersson and Holmdahl, Eur J Immunol 20:1061-1066, 1990, preferably the assay is carried out using the methodology used in the Examples herein.

The peptides of the invention are of use in therapy without modification but alternatively the peptides may be modified, for example they may be conjugated, e.g. bound covalently, to delivery systems, for example to mucosal binding structures which include the cholera β toxin which assists absorption in the intestine.

The peptides of the present invention may be used in the treatment, prophlaxis or diagnosis of autoimmune conditions and the terms 'therapy' and 'treatment' as used herein should be taken also to include prophylaxis and diagnosis.

A peptide of the present invention is to be taken to be an isolated peptide in the sense that peptides of the present invention do not include peptides present in an organism. Peptides of the present invention may be either isolated from a naturally or recombinantly produced peptide or protein or may be chemically synthesised as herein described.

The compounds may be administered at a dosage from about 10 μg to 10 mg per day either as a single dose or in divided doses 2 to 4 times per day. Thus unit doses comprise from 2.5 μg to 10 mg of a compound according to the invention. The compounds may be administered intranasally in the form of solutions, suspensions, HFA aerosols and dry powder formulations, e.g. Turbuhaler® formulations; or systemically, e.g. by oral

administration in the form of tablets, pills, capsules, syrups, powders or granules, or by parenteral administration in the form of sterile parenteral solutions or suspensions, or by rectal administration in the form of suppositories.

The compounds of the invention may be administered on their own or as a pharmaceutical composition comprising the compound of the invention in combination with a pharmaceutically acceptable diluent, adjuvant or carrier. Particularly preferred are compositions not containing material capable of causing an adverse, e.g. an allergic, reaction.

Dry powder formulations and pressurized HFA aerosols of the compounds of the invention may be administered by nasal inhalation. For inhalation the compound is desirably finely divided. The finely divided compound preferably has a mass median diameter of less than 10 μm, and may be suspended in a propellant mixture with the assistance of a dispersant, such as a C_8-C_{20} fatty acid or salt thereof, (e.g. oleic acid), a bile salt, a phospholipid, an alkyl saccharide, a perfluorinated or polyethoxylated surfactant, or other pharmaceutically acceptable dispersant.

The compounds of the invention may also be administered by means of a dry powder inhaler. The inhaler may be a single or a multi dose inhaler, and may be a breath actuated dry powder inhaler.

One possibility is to mix the finely divided compound with a carrier substance, e.g. a mono-, di- or polysaccharide, a sugar alcohol or another polyols. Suitable carriers are sugars, e.g. lactose, glucose, raffinose, melezitose, lactitol, maltitol, trehalose, sucrose, mannitol; and starch. Alternatively the finely divided compound may be coated by another substance. The powder mixture may also be dispensed into hard gelatine capsules, each containing the desired dose of the active compound.

The pharmaceutical composition comprising the compound of the invention may conveniently be tablets, pills, capsules, syrups, powders or granules for oral administration;

sterile parenteral solutions or suspensions for parenteral administration or suppositories for rectal administration.

For oral administration the active compound may be admixed with an adjuvant or a carrier, e.g. lactose, saccharose, sorbitol, mannitol, starches such as potato starch, corn starch or amylopectin, cellulose derivatives, a binder such as gelatine or polyvinylpyrrolidone, and a lubricant such as magnesium stearate, calcium stearate, polyethylene glycol, waxes, paraffin, and the like, and then compressed into tablets. If coated tablets are required, the cores, prepared as described above, may be coated with a concentrated sugar solution which may contain e.g. gum arabic, gelatine, talcum, titanium dioxide, and the like. Alternatively, the tablet may be coated with a suitable polymer dissolved in a readily volatile organic solvent.

For the preparation of soft gelatine capsules, the compound may be admixed with e.g. a vegetable oil or polyethylene glycol. Hard gelatine capsules may contain granules of the compound using either the above mentioned excipients for tablets, e.g. lactose, saccharose, sorbitol , mannitol, starches, cellulose derivatives or gelatine. Also liquid or semisolid formulations of the drug may be filled into hard gelatine capsules.

Liquid preparations for oral application may be in the form of syrups or suspensions, for example solutions containing the compound, the balance being sugar and a mixture of ethanol, water, glycerol and propylene glycol. Optionally such liquid preparations may contain colouring agents, flavouring agents, saccharine and carboxymethylcellulose as a thickening agent or other excipients known to those skilled in art.

The invention is now illustrated by the following Examples which should not be interpreted as limiting the present invention.

Example 1

The CII peptide chains listed in Table 1 each consisting of 15 amino acids were synthesised using an SMPS 350 automated synthesiser (Zinsser, Frankfurt/Main, Germany) using known Fmoc chemistry (see Atherton and Sheppard, Solid Phase Peptide Synthesis - A Practical Approach. IRL Press, Oxford). Each peptide was then acetylated at the N-terminus and amidated at the C- terminus. The quality of the peptides was assessed by HPLC and mass spectroscopy of a sample of the peptides confirmed the expected molecular weight.

Table 1

Peptide	Amino acid residues	Peptide	Amino acid residues
110-124	VKG **ESG SPG ENG** SPG	635-649	FAG **PPG ADG QPG** AKG
140-154	AAG **ARG NDG QPG** PAG	641-655	ADG **QPG AKG DQG** EAG
161-175	GPG FPG APG AKG EAG	665-679	PSG APG PQG PTG VTG
170-184	**APG AKG EAG** PTG ARG	677-691	PQG **PTG VTG PKG** ARG
185-199	PEG **AQG SRG EPG** NPG	701-715	AAG **RVG PPG ANG** NPG
203-217	**PAG ASG NPG** TDG IPG	707-721	PPG **ANG NPG PAG** PPG
209-223	NPG **TDG IPG AKG** SAG	740-754	RAG **DPG LQG PAG** APG
218-232	AKG **SAG APG IAG** APG	749-763	PAG **APG EKG EPG** DDG
224-238	APG **IAG APG FPG** PRG	770-784	PPG **PQG LAG QRG** IVG
230-244	APG **FPG PRG PPG** PQG	779-793	QPG IVG LPG QPG ERG
338-352	LAG **PKG ANG DPG** RPG	806-820	KQG **APG ASG DRG** PPG
353-367	EPG **LPG ARG LTG** RPG	821-835	PVG PPG LTG PAG EPG
365-379	RPG **DAG PQG KVG** PSG	857-871	ETG **ALG APG APG** PPG
593-607	PPG **PAG ANG EKG** EVG	881-895	PTG **KQG DRG EAG** AQG
614-628	STG **ARG APG EPG** ETG		

The portions of the amino acid which are shown in bold were found to be the core sequences, i.e. the parts of the amino acids which were bound most strongly, by comparing the binding strengths of structurally closely related peptides.

Example 2

Mouse CII was extracted from xiphisterna by pepsin digestion using known techniques and then further purified by salt precipitation. Rat CII was purified from the Swarm chondrosarcoma again using known techniques. The collagens were dissolved in 0.1 M acetic acid. Collagen for use in restimulating primed lymph node cells was denatured by incubating at 56°C for 30 minutes.

Example 3

To test proliferative responses and cytokine release in drained lymph node cells, male (B10.Q X DBA/1) F1 mice, 7-10 weeks of age, were immunised in each hind foot pad with 50 µg mouse CII or synthetic peptide emulsified in CFA (containing H37Ra, Difco, Detroit, MI). Arthritis was induced in the same mice when they were 7 to 10 weeks old, by immunising the mice at the base of the tail with 100µg of rat CII emulsified CFA as prepared in Example 2.

After 5 weeks, mice were boosted with 50µg of CII emulsified in a 1:1 ratio by weight with IFA (DIFCO, Detroit, MI). Lymph node cells drained from severely arthritic joints from these mice were then removed and pooled to test reactivity to the panel of mouse CII peptides prepared in Examples 1 in a proliferation assay in the following way.

Cytokine release was assayed from 50 µl supernatants of primary cultures of mouse CII primed lymph node cells were restimulated in vitro with the panel of CII peptides prepared according to Example 1 using IFN-γ and IL-4 minikits (Endogen, Cambridge, MA).

The results are shown in Table 2.

Table 2

Peptide	SI	IFN-γ Release (ng/ml)	Peptide	SI	IFN-γ Release (ng/ml)
110-124	2.2	2.2	635-649	2.7	0.4
140-154	2.1	1.5	641-655	2.0	0.9
161-175	3.1	4.2	665-679	3.5	1.5
170-184		1.5	677-691	2.2	2.9
185-199	4.1	1.9	701-715	18.0	2.5
203-217	3.6	6.5	707-721	19.4	5.8
209-223	5.2		740-754	2.8	2.4
218-232	3.9	2.3	749-763	4.8	1.0
224-238	5.0	4.4	770-784	2.7	1.4
230-244	3.9	5.8	779-793	3.9	
338-352	4.4	2.0	806-820	2.3	
353-367	3.1	1.5	821-835		1.8
365-379	2.8		857-871	2.4	0.3
593-607	2.9	0.7	881-895	2.6	0.6
614-628	2.7	2.3			

Example 4

Lymph node cells from mice primed with mouse CII were depleted of either T cells or B cells by passage through a magnetically activated cell sorter (MACS) using super-paramagnetic microbeads conjugated with monoclonal rat anti-mouse L3T4 (CD4) antibodies or rat anti-mouse B220 (CD45R) (both from Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) as recommended by the manufacturer except that PBS containing 2% FCS, 5 mM EDTA, 50 μM 2-ME and 10 mM HEPES was substituted for PBS/BSA buffer. The fractions were analysed on a FACScan flow cytometer (Becton Dickinson) using FITC-conjugated anti-mouse-CD3-ε for staining T-cells and FITC-conjugated anti-mouse-κ-light-chain for B cells (Pharmigen, San Diego, CA). The cells were washed three times with DMEM medium without serum after separation and then put into proliferation assays using the method described in Example 3. Enriched CD4+ T cells were mixed together with spleen cells from spleens from syngeneic mice as antigen presenting cells in a ratio of 1:2 by weight. AP cells are used in the form of a single cell suspension from spleens treated with 0.84% NH_4Cl at pH 7.4 to lyse red blood cells.

Table 3

Cell Type	CPM $x10^{-3}$
Unfractionated	13.6
CD4+ Enriched	21.4
CD4+ Depleted	0.3
CD45R+ Depleted	12.5
CD45R+ Enriched	0.4

SEQUENCE LISTING

SEQUENCE ID No. 1: ESG SPG ENG

SEQUENCE ID No. 2: PPG ADG QPG

SEQUENCE ID No. 3: ARG NDG QPG

SEQUENCE ID No. 4: QPG AKG DQG

SEQUENCE ID No. 5: APG AKG EAG

SEQUENCE ID No. 6: PTG VTG PKG

SEQUENCE ID No. 7: AQG SRG EPG

SEQUENCE ID No. 8: RVG PPG ANG

SEQUENCE ID No. 9: PAG ASG NPG

SEQUENCE ID No. 10: ANG NPG PAG

SEQUENCE ID No. 11: TDG IPG AKG

SEQUENCE ID No. 12: DPG LQG PAG

SEQUENCE ID No. 13: SAG APG IAG

SEQUENCE ID No. 14: APG EKG EPG

SEQUENCE ID No. 15: IAG APG FPG

SEQUENCE ID No. 16: PQG LAG QRG

SEQUENCE ID No. 17: FPG PRG PPG

SEQUENCE ID No. 18: PKG ANG DPG

SEQUENCE ID No. 19: APG ASG DRG

SEQUENCE ID No. 20: LPG ARG LTG

SEQUENCE ID No. 21: DAG PQG KVG

SEQUENCE ID No. 22: ALG APG APG

SEQUENCE ID No. 23: PAG ANG EKG

SEQUENCE ID No. 24: KQG DRG EAG

SEQUENCE ID No. 25: ARG APG EPG

SEQUENCE ID No. 26: VKG ESG SPG ENG SPG

SEQUENCE ID No. 27: FAG PPG ADG QPG AKG

SEQUENCE ID No. 28: AAG ARG NDG QPG PAG

SEQUENCE ID No. 29: ADG QPG AKG DQG EAG

SEQUENCE ID No. 30: APG AKG EAG PTG ARG

SEQUENCE ID No. 31: PQG PTG VTG PKG ARG

SEQUENCE ID No. 32: PEG AQG SRG EPG NPG

SEQUENCE ID No. 33: AAG RVG PPG ANG NPG

SEQUENCE ID No. 34: PAG ASG NPG TDG IPG

SEQUENCE ID No. 35: PPG ANG NPG PAG PPG

SEQUENCE ID No. 36: NPG TDG IPG AKG SAG

SEQUENCE ID No. 37: RAG DPG LQG PAG APG

SEQUENCE ID No. 38: AKG SAG APG IAG APG

SEQUENCE ID No. 39: PAG APG EKG EPG DDG

SEQUENCE ID No. 40: APG IAG APG FPG PRG

SEQUENCE ID No. 41: PPG PQG LAG QRG IVG

SEQUENCE ID No. 42: APG FPG PRG PPG PQG

SEQUENCE ID No. 43: LAG PKG ANG DPG RPG

SEQUENCE ID No. 44: KQG APG ASG DRG PPG

SEQUENCE ID No. 45: EPG LPG ARG LTG RPG

SEQUENCE ID No. 46: RPG DAG PQG KVG PSG

SEQUENCE ID No. 47: EXG ALG APG APG PPG

SEQUENCE ID No. 48: PPG PAG ANG EKG EVG

SEQUENCE ID No. 49: PTG KQG DRG EAG AQG

SEQUENCE ID No. 50: STG ARG APG EPG ETG

SEQUENCE ID No. 51: AAG RVG PPG ANG NPG

SEQUENCE ID No. 52: PPG ANG NPG PAG PPG

Claims

1. An isolated peptide having an amino acid sequence of formula (I):

$$A_1\text{-Xaa-Gly-}A_4\text{-}A_5\text{-Gly-}A_7\text{-Xaa-Gly} \qquad (I)$$

wherein;

A_1 represents an amino acid residue with an aromatic or aliphatic side chain,

A_4 represents an asparagine or arginine residue or an amino acid residue with an aromatic or aliphatic side chain,

A_5 represents any naturally occurring amino acid,

A_7 represents an amino acid with a negatively charged residue,

Xaa represents any amino acid residue, and

Gly represents a glycine residue.

2. An isolated peptide comprising the amino acid sequence of any of the peptides claimed in claim 1.

3. A peptide according to claim 2 which contains between 10 and 15 amino acids.

4. A peptide according to claim 1 wherein A_5 represents a lysine or arginine residue and A_7 represents a glutamic acid residue.

5. An isolated peptide having any one of the following sequences:

ESG SPG ENG,

PPG ADG QPG,

ARG NDG QPG,

QPG AKG DQG,

APG AKG EAG,

PTG VTG PKG,
AQG SRG EPG,
RVG PPG ANG,
PAG ASG NPG,
ANG NPG PAG,
TDG IPG AKG,
DPG LQG PAG,
SAG APG IAG,
APG EKG EPG,
IAG APG FPG,
PQG LAG QRG,
FPG PRG PPG,
PKG ANG DPG,
APG ASG DRG,
LPG ARG LTG,
DAG PQG KVG,
ALG APG APG,
PAG ANG EKG,
KQG DRG EAG, or
ARG APG EPG (SEQ IDs Nos 1 to 25 respectively).

6. An isolated peptide comprising the amino acid sequence of any of the peptides claimed in claim 3.

7. An isolated peptide having any one of the following sequences:

VKG ESG SPG ENG SPG;
FAG PPG ADG QPG AKG;
AAG ARG NDG QPG PAG;
ADG QPG AKG DQG EAG;

APG AKG EAG PTG ARG;
PQG PTG VTG PKG ARG;
PEG AQG SRG EPG NPG;
AAG RVG PPG ANG NPG;
PAG ASG NPG TDG IPG;
PPG ANG NPG PAG PPG;
NPG TDG IPG AKG SAG;
RAG DPG LQG PAG APG;
AKG SAG APG IAG APG;
PAG APG EKG EPG DDG;
APG IAG APG FPG PRG;
PPG PQG LAG QRG IVG;
APG FPG PRG PPG PQG;
LAG PKG ANG DPG RPG;
KQG APG ASG DRG PPG;
EPG LPG ARG LTG RPG;
RPG DAG PQG KVG PSG;
ETG ALG APG APG PPG;
PPG PAG ANG EKG EVG;
PTG KQG DRG EAG AQG; or
STG ARG APG EPG ETG (SEQ IDs Nos 26 to 52 respectively).

8. An isolated peptide which is a 10 to 14 amino acid peptide fragment of any one of the peptides claimed in claim 7.

9.A peptide according to any one of the preceding claims which binds to T-cells with a binding strength of at least 2 in an IFN-γ release assay.

10. A peptide according to any one of the preceding claims which binds to T-cells with a stimulation index of at least 3.

11. A peptide according to any one of the preceding claims wherein the sequence of the peptide is present in the sequence of collagen II between positions 110-239, 338-379 or 587-895.

12. A peptide according to claim 11, wherein the sequence of the peptide is present in the 701-721 region of collagen II.

13. An isolated peptide according to any of the preceding claims, for use in medical therapy.

14. A peptide according to claim13, wherein the medical therapy comprises the induction of tolerance.

15. Use of an isolated peptide according to any of the preceding claims, in the manufacture of a medicament for use in the therapy of an autoimmune condition.

16 Use according to claim 10, wherein the autoimmune condition is rheumatoid arthritis.

17. A pharmaceutical composition comprising a peptide according to any of claims 1 to 12 in association with a pharmaceutically acceptable carrier or diluent therefor.

18. A method of treating a human or animal suffering or liable to suffer from an autoimmune condition, which method comprises supplying the human or animal with a therapeutically effective amount of a peptide according to any one of claims 1 to 12 or of a composition according to claim 17.

19. A peptide comprising a T-cell epitope specific to collagen II which peptide comprises at least nine amino acids having the same sequence as and selected consecutively from the 110-239, 338-379 and 587-895 portions of the sequence of collagen II wherein each amino

acid is optionally replaced by a functionally equivalent amino acid and wherein the peptide is of formula

A_1-Xaa-Gly-A_4-A_5-Gly-A_7-Xaa-Gly (I)

wherein;

A_1 represents an amino acid residue with an aromatic or aliphatic side chain,

A_4 represents an asparagine or arginine residue or an amino acid residue with an aromatic or aliphatic side chain,

A_5 represents any naturally occurring amino acid,

A_7 represents an amino acid with a negatively charged residue,

Xaa represents any amino acid residue, and

Gly represents a glycine residue.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00128

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 7/06, C07K 14/78, A61K 38/08, A61K 38/39
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, REGISTRY, WPI, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Immunology, Volume 86, 1995, H. Miyahara et al, "Identification and characterization of a major tolerogenic T-cell epitope of type II collagen that suppresses arthritis in B10.RIII mice",", page 110- page 115, the summary; page 111, left c olumn, paragraph 2	1-19
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X	WO 9620950 A2 (IMMULOGIC PHARMACEUTICAL CORPORATION), 11 July 1996 (11.07.96), the abstract; the claims; page 4, lines 28-30; page 29, SEQ ID NO:2	1-19
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 May 1998	19-05-1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ional application No.
PCT/SE 98/00128

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	International Immunology, Volume 8, No 5, 1996, Sho Matsushita et al, "HLA-DQ-binding peptide motifs. I. Comparative binding analysis of typoe II collagen-derived peptides to DR and DQ molecules of rheumatoid arthritis-susceptible and non-susceptible haplotypes", page 757 - page 764; Table 2	1-19
	--	
X	Eur. J. Immunol, Volume 23, 1993, Grace Ku et al, "Prevention of experimental autoimmune arthritis with a peptide fragment of type II collagen" page 591 - page 599	1-19
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X	Eur. J. Immunol, Volume 22, 1992, Erik Michaëlsson et al, "Identification of an immunodominant type-II collagen pepteide recognized by T cells in H-2q mice: self tolerance at the level of determinant selection" page 1819 - page 1825	1-19
	--	
X	JP 8-151396 A (OL), 11 June 1996 (11.06.96), page 7	1-19
	--	
A	Eur. J. Immunol, Volume 24, 1994, Xiao-Ming Gao et al, "Collagen specific cytotoxic T lymphocyte responses in patients with ankylosing spondylitis and reactive arthritis" page 1665 - page 1670	1-19
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00128

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 18
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 18 is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound. (c.f. PCT rule 39.1 (iv)).

2. [X] Claims Nos.: 1-2
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
See next page

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.
[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/04/98

International application No.
PCT/SE 98/00128

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9620950 A2	11/07/96	AU 4695296 A	24/07/96
JP 8-151396 A	11/06/96	NONE	